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Effective nitrogen removal from onsite wastewater using a sequencing aerated biofilm reactor

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ABSTRACT

A novel sequencing aerated biofilm reactor (SABR) was designed for effective nitrogen removal from onsite wastewater with a footprint 10 times smaller than conventional drainfield and a high hydraulic capacity. The study examined the effect of aeration pattern, wastewater strength, and carrier type on N-removal performance. Over 93 % COD removal and > 80 % TN-removal was achieved in the SABR integrated with a polishing unit (10 % woodchips, v/v) at the optimized aeration pattern (4-h pre-anoxic, 7 aeration cycles of 20 min aeration per hour at 1 L-air/min airflow rate, 1 h post-anoxic). Carriers' shape and surface area did not impact SABR's N-removal performance (31.8 % vs. 28.2 %). The optimal operation conditions obtained in bench-scale SABR tests were pilot tested with the 10 % woodchip polishing unit. Efficient TN-removal (72.4 %) was achieved with low effluent TN concentrations (6.5 ± 3.9 mg-N/L) by the pilot SABR. Ammonium (NH_4^+) was the predominant N-species (5.5 ± 6.0 mg-N/L) in the final effluent while NO_x^- was constantly below the detection limit (< 0.05 mg-N/L). Quantitative PCR analysis of functional genes involved in N-removal (*amoA*-AOA, *amoA*-AOB, *nirS*, *nirK* and *nosZ*) were comparable for bench-scale and pilot-scale SABRs and revealed higher abundance of *amoA*-AOB (> 4 orders of magnitudes higher than *amoA*-AOA) and *nirS* (*nirK*/*nirS*: 0.2–1.8 × 10^{-2}), suggesting *amoA*-AOB and *nirS* may serve as biomarkers to monitor the system performance. Collectively, the results suggest that SABR offers a versatile approach to treat wastewater at various strengths and is applicable in areas with space constraints, shallow groundwater, and sensitive water bodies.

1. Introduction

Decentralized wastewater is one of the largest sources of excess nitrogen (N) in shallow groundwater [1–3]. Conventional onsite wastewater treatment systems (OWTSs) consisting of septic tanks and leachfields, are not designed to remove N [4]. They offer a basic wastewater treatment where particles settle to the septic tanks' bottom and undergo partial degradation. Consequently, septic tank effluent (STE), which contains high levels of N, is then further dispersed in a leachfield/cesspools from where it leaks into the surrounding soil and into the aquifer. Only 1– 30 % of total nitrogen (TN) is removed by conventional OWTSs, a statistic unchanged since their invention a century ago [1–3,5–7].

N-removal in an OWTS could be achieved by: i) physical and/or chemical processes that require a high level of operational attention, substantial chemical, and energy costs, and may generate large volumes of residuals (e.g. membrane separation, ion exchange, evaporation, etc.) [8–10]; ii) engineered biological nitrification/denitrification processes requiring mechanical aeration [11]; and iii) soil-based filtration treatment, such as wetlands, subsurface wastewater infiltration systems (SWIS) or nitrogen removing biofilters (NRBs) [12–14]. Among these, soil-based filtration provides a cost-effective enhanced N-removal, integrating easily with conventional OWTS after septic tanks. Current NRBs rely on the autotrophic nitrification and heterotrophic denitrification processes. NRBs requires the system design to comprise a conservative depth (90 cm) with a modest loading rate (~0.024–0.048 $m^3/m^2/d$), leading to a large footprint (15.85 m × 5.28 m for a 4 bedroom house) [12]. Implementing this system in populated areas with shallow groundwater tables or limited land space is difficult. Additionally, the woodchip denitrification layer's performance is temperature-sensitive,

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leading to incomplete denitrification in winter. The implementation of a polishing unit following the lignocellulose treatment unit has been proposed to mitigate N levels discharged into the environment [15–17]. Yet, these strategies necessitate the incorporation of supplementary materials (such as elemental sulfur, zero-valent iron), which increase construction cost and contribute to secondary contamination, such as sulfate and ferric iron, in the groundwater [16].

Studies have demonstrated that a lack of oxygen negatively affects the N-removal in filtration-based OWTSs, like wetlands, SWISs [18–20], and intermittent baffles bioreactors (iBBRs) [21]. Mechanical aeration has been employed in wetlands [22], advanced treatment units (ATUs) [23], and filtration systems [24–26] resulting in elevated dissolved oxygen (DO) and promoting the nitrification rather than TN removal, due to the unfavorable redox conditions necessary for denitrification. Complete denitrification requires 2.86 g BOD per gram of nitrate [27]. The lack of available carbon in onsite wastewater becomes the primary limiting factor for TN removal [27–29]. Intermittent aeration, which alternates aerobic/anoxic conditions for nitrification and denitrification, has been shown to increase TN removal efficiency, reducing operating costs [24,29] and leveraging the carbon in raw wastewater for denitrification. On the other hand, previous studies suggested that an external carbon source was crucial for complete denitrification [30]. The minimum carbon to nitrogen ratio (C: N) required was established as 3.5–4.5 g COD gr^{-1} N, with the range of 6–11 g COD gr^{-1} N deemed optimal for N-removal [31,32]. However, STE often contains a low C: N ratio (<8.0) [32], resulting in challenges in denitrification [33–35]. To enhance N-removal performance, the addition of external carbon sources such as slow carbon-releasing lignocellulose (e.g. woodchips) in the passive OWTSs was proved to effectively improve the activity of denitrifying microorganisms and enhanced the TN removal efficiency [12,32,36,37].

A sequencing aerated biofilm reactor (SABR) merges the benefits of a sequencing batch reactor with those of a biofilm process. By alternating oxic and anaerobic conditions, it facilitates simultaneous nitrification, BOD removal, and denitrification within a unified biofilm reactor. This configuration, robust in nature, exhibits reduced sensitivity to the dynamic composition and flow patterns of onsite wastewater [38]. The design not only guarantees efficient and consistent treatment but also offers the additional benefit of requiring minimal footprint. In this work, we developed a novel SABR for efficient N-removal from STE. The SABR system has a small footprint (One-tenth the size of the conventional drainfield) that can accommodate high hydraulic loadings, with a minimal external carbon amendment in the polishing unit and without alkalinity amendment. The impact of aeration strategy, STE loadings, biofilm carrier type, and influent composition, on N-removal were systematically studied. The results of this research will provide valuable insights into the design and optimization of next-generation OWTSs for efficient N-removal.

2. Materials and methods

2.1. System design and operation

A bench-scale SABR was constructed at the Wastewater Research and Innovation Facility (WRIF) at Stony Brook University, NY. Briefly, a clear acrylic column with an effective volume of three liters was equipped with two optical oxygen sensors (FireSting O2, Pyroscience GmbH) at 7.5 cm and 15 cm from the bottom of the reactor and a temperature (T) sensor (FireSting O2, Pyroscience GmbH) (Fig. 1(a)). The SABR-J-WC was filled with 50 % Jaegar carriers and 50 % oak woodchips (Table S1). The SABR-J was filled with 100 % Jaegar carriers, while the following polish column was filled with 10 % woodchips and 90 % Jaegar carriers (SABR-Polish). The SABR-K was packed with 100 % Kaldnes-K1 carriers. Air was supplied to the reactors from the bottom using timer-operated air pumps (Hydrofarm Active Aqua pump). A multi-channel peristaltic pump transferred STE and effluent to and from the SABRs (Ismatec Reglo ICC Digital Peristaltic Pump, Cole-Parmer®). After 20-month STE treatment, the SABR was scaled-up to SABR-Pilot-K (Fig. 1(b)) using a plastic barrel (400 L) packed with Kaldnes-K1-micro carriers. Two optical oxygen sensors (25 and 50 cm from the bottom) and a T-sensor (FireSting O2, Pyroscience GmbH) were installed inside the SABR-Pilot-K. A subsequent 400 L polishing SABR (Pilot-Polish) was filled with 10 % woodchips and 90 % carriers, was fully saturated and operated as an up-flow bioreactor. Air was applied to the reactors from the bottom using air pump (ColeParmer Air Admiral vacuum pump). Two Masterflex® L/S® Digital Drive peristaltic pumps transported STE to the SABR-Pilot-K and Pilot-polish.

2.2. Experimental stages and aeration pattern

Total of seven stages of bench-scale SABR experiments were conducted (Table 1). At the start of each 12-h hydraulic cycle, the SABR was fed with real STE using peristaltic pumps at a flow rate of 35 mL/min for 22 min, then a sequence of anoxic and aerobic conditions were introduced by timer operating air pumps (Table 1). At the end of the 12-h hydraulic cycle, the effluent was withdrawn at 35 mL/min for 22 min.

At stage 1, intermittent aeration (20 min air/h for 10 h) was applied to SABR-J and SABR-J-WC at 1 L-air/min. At stage 2, SABR-J-WC was terminated due to its poor N-removal performance. The aeration pattern was adjusted to 40 min idle: 20 min aeration per hour at 0.5 L-air/min. The aeration was limited to the first 2 h to maintain extended anoxic conditions for denitrification. At stage 3, a pre-anoxic period (3 h) was

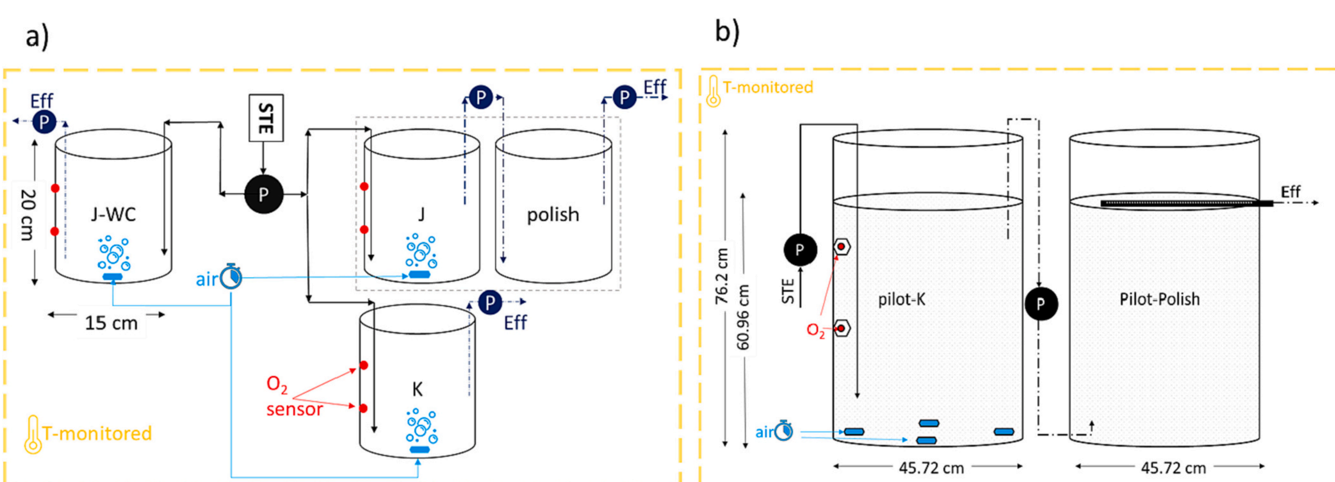

Fig. 1. Schematic of (a) bench-scale SABR -J-WC, –J, —K and -Polish (b) SABR-Pilot-K and Pilot-Polish.

Table 1
Selected operation conditions for the bench-scale SABR treating real STE.

Stage	C: N	Airflow rate (L min^{-1})	Pre-anoxic period (hr)	Aeration pattern	Anoxic (hr)	Duration (day)
1	6.4	1	no	20 min air: 40 min idle	2	41*-130
2	8.7	0.5	no	40 min idle: 20 min air	10	131–174
3	8.9	0.5	3	20 min air: 40 min idle	2	175–211
4	3.3	1	3	20 min air: 40 min idle	2	211–317
5	3.5	1	4	20 min air: 40 min idle	1	318–378
6	6.7	1	4	20 min air: 40 min idle	1	379–473
7**	5.4	1	4	20 min air: 40 min idle	1	476–621

* First 40 days were initial star-up period.
** The HRT of stage 1–6 was 2 days, the HRT at stage 7 was 3 days.

applied to SABR-J when influent was added, and the aeration cycles were adjusted to 20 min air: 40 min idle per hour at 0.5 L-air/min to enhance denitrification. At stage 4, the airflow rate was increased to 1 L-air/min to enhance nitrification. At stage 5, the pre-anoxic condition was extended to 4 h and a SABR-Polish was added to SABR-J with same HRT. To compare the SABR performance using different types of carriers, SABR-K was constructed and operated under the same hydraulic and aeration conditions as SABR-J. At stage 6, synthetic wastewater was prepared and mixed with STE to increase the strength of the influent (COD: 376.1 mg/L and TN: 72.1 mg-N/L, C: N: 6.7). At stage 7, the hydraulic retention time (HRT) of both SABRs was increased from 2 to 3 days to improve the COD removal and nitrification in response to high strength STE. To validate the optimal hydraulic and aeration patterns identified at bench-scale tests, the operation parameters acquired at stage 5 were applied in the pilot-scale for 145 days (Table 1).

2.3. Sample collection and analytical methods

STE and SABR effluent samples were collected weekly, acidified with concentrated sulfuric acid (18 M H_2SO_4), and stored at 4 °C for analysis. pH was measured in situ by a Sension+ MM150 multi-parameter meter (Hach, Colorado, USA). DO, and T data were collected using fiber-optic oxygen and T sensors. Ammonia and NO_x^- (combination of nitrite and nitrate) were measured using Lachat's Quikchem® 8500 Series 2 Flow Injection Analysis System as described previously [13]. In brief, NH_4^+ was measured by the salicylate method and NO_x^- measurement was conducted by the cadmium reduction method. TN and COD were measured based on the persulfate digestion method (Method 10,071) and the USEPA dichromate digestion method 8000 (Standard Method 5220 D) using a HACH Spectrophotometer (DR6000, HACH). The digestion process was performed using the DRB200 digester (HACH).

2.4. Microbial analysis

Carriers and woodchips samples were collected at the end of stages 4, 5, and 7 from the bench-scale SABRs, and at the beginning and the end of the SABR-Pilot operation. Biofilm was extracted from biocarriers preserved in RNase-free PBS solution (pH 7.4, Invitrogen™) through ultrasonication. Suspended media (MLSS and biofilm) was centrifuged (10 min at 10,000g), and the supernatant was decanted. Cell pellets were preserved at −80 °C for further analysis. Meanwhile, approximately 0.25 g of woodchips were subjected to the collection protocol of the Powerlyzer power soil DNA Isolation Kit (Qiagen) to obtain the biofilm. Genomic DNA was extracted from the carriers and liquid samples using the Powerlyzer power soil DNA kit (Qiagen) according to the manufacturer's instructions. The DNA extracted samples were stored at −80 °C freezer until further analysis. Abundance of the functional species, including total bacteria (16S rRNA), nitrifiers (*amoA*-AOA and *amoA*-AOB), and denitrifiers (*nirS*, *nirK*, and *nosZ*) were measured via qPCR analysis on a StepOne Plus Real-time PCR system (Applied Biosystems, USA) (Table S2). Each qPCR assay contains a standard curve (a serial dilution of the plasmids) in triplicate, independent triplicates for each sample, and triplicate no template controls (NTCs) [36]. To ensure comparability for the gene abundance for MLSS and biofilm samples, gene abundance in the biofilm (gene copies per biocarrier) was normalized to a 1 mL volume of the SABR.

2.5. Statistical analysis

Two sample *t*-test was used to evaluate whether new process/treatment is superior to a current process/treatment when data were normally distributed. The nonparametric Mann-Whitney test was used to check if operation condition change statistically changed the system performance when data were not normally distributed [39–42]. Analyses were performed in OriginLab 2020 (OriginLab, Northampton, MA).

3. Results and discussions

3.1. Impact of woodchips amendment on N-removal by the SABR

Woodchips were amended in SABR-J-WC during the system start-up period to facilitate denitrification (Fig. 1(a)). However, constant low DO (<0.2 mg/L) was observed throughout the 120 days of operation. In SABR-J-WC, DO was primarily used to degrade the organic matter in the STE, as well as the labile carbon in woodchips. As a result, 51 % COD removal from the STE was achieved, with the final effluent containing 111.1 ± 43.2 mg/L COD (Fig. S1(a)). NH_4^+-N (32.5 ± 11.0 mg-N/L) was the dominant N-species in the final effluent, while NO_x^--N concentration was <0.5 mg-N/L leading to limited to no N-removal (Fig. S1(b) and (c)). Correspondingly 81 % COD an overall 24.3 % TN removal were achieved by SABR-J from the STE (Fig. S1(a) and (b)). SABR-J filled with only carriers achieved an average of 71 % nitrification with sufficient DO (0–4 mg/L), and NO_x^- (16.9 ± 10.9 mg-N/L) was the major N-species in the final effluent (Fig. 2(c)). These results show that directly adding a slow-releasing carbon source to the primary SABR wasn't effective for enhancing N-removal.

3.2. N-removal by bench-scale SABRs treating real STE

3.2.1. Aeration pattern impact

In absence of pre-anoxic phase (stage 1), DO levels at the top and the bottom of the reactor were comparable at 2–3 mg/L during aeration (1 L-air/min for 10 h) and decreased to <0.2 mg/L during the post anoxic period (Fig. S2). SABR-J achieved 79.1 % COD removal, and the average effluent TN was 26.8 ± 5.4 mg-N/L. When the aeration rate decreased to 0.5 L-air/min at stage 2, DO concentration was between 2 and 4 mg/L during aeration and then decreased to <0.2 mg/L during the extended post-anoxic period (Fig. S2). SABR-J accomplished 73.5 % COD removal and the average effluent TN was 29.0 ± 8.6 mg-N/L. A pre-anoxic period was introduced to the SABR to facilitate denitrification at stage 3. DO level in the SABR was <1 mg/L at the bottom and 1–2 mg/L at the top sensors. COD removal of 73.5 % was achieved and effluent TN was an average of 32.7 ± 4.3 mg-N/L.

At stage 4, the airflow rate was increased to 1 L-air/min to enhance the DO levels to maintain sufficient nitrification performance. A time course analysis of one hydraulic cycle (12h) was performed to understand the N-removal mechanisms (Fig. S3). DO increased to 4 mg/L at the top, and 3 mg/L at the bottom during each aeration event and gradually decreased to <0.2 mg/L during the idle period. After the first 20 min of adding STE, the measured values of TN and COD were 28 mg-

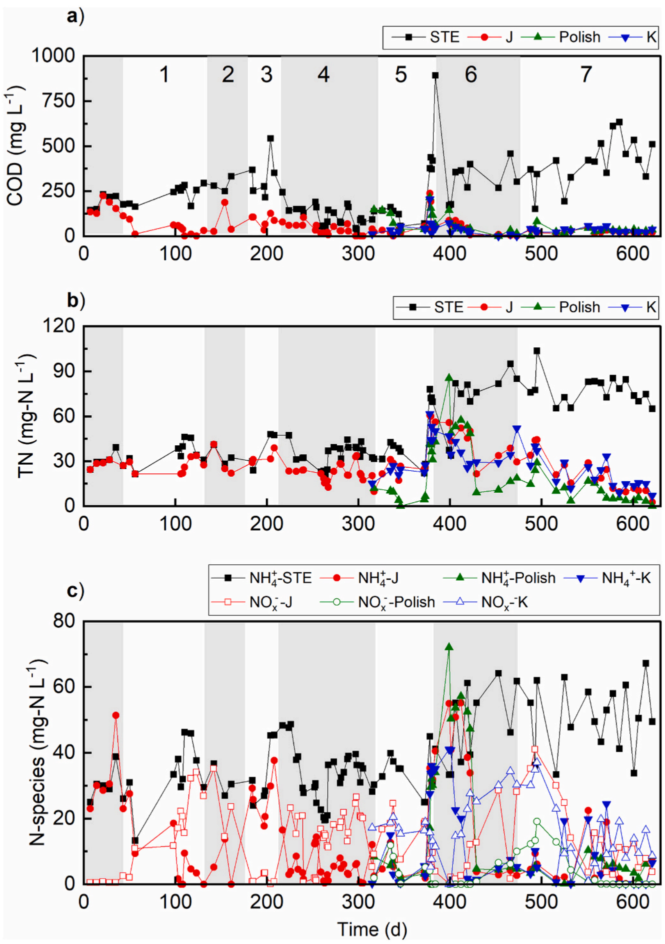

Fig. 2. (a) COD, (b) TN, and (c) N-species concentrations of SABR-J, polishing unit for SABR-J and SABR-K treating STE at different operational stages.

N/L and 74 mg/L, respectively. COD decreased to 37 mg/L and NO_x^- from 13 mg-N/L to 8.5 mg-N/L during the initial 3-h pre-anoxic condition. Subsequently, ammonia decreased from 12 mg-N/L to 4 mg-N/L after consecutive aeration. The results indicated denitrification took place mainly during the pre-anoxic time while DO was low (<0.2 mg/L) and COD in STE was used (>35 mg/L) as a carbon source. The DO saturation constants of nitrifiers (ammonia-oxidizing bacteria-AOB and nitrite-oxidizing bacteria-NOB) in activated sludge ranged from 0.25 to 0.5 mg/ L (AOB) and from 0.34 to 2.5 mg/ L (NOB) [43]. It has been shown that extended non-aeration periods (2−3*h*) can impact NOBs and cause lagged nitratation after the anoxic state is removed [44,45]. During the pre-anoxic condition, SABR remained anoxic (DO <0.2 mg/ L) before intermittent aeration was applied due to COD degradation and nitrification. The improved aerobic condition through intermittent aeration was suitable for the growth of bacteria in stage 4 compared to previous stages (stages 1–3) [28].

At stage 5, the pre-anoxic period was increased to 4 h to investigate if pre-anoxic conditions cause additional denitrification. An average of 31.8 % TN-removal and 76.0 % COD removal were achieved from STE with a low C: N ratio (3.5: 1). COD removal, TN-removal and nitrification achieved by SABR-J at stage 5 were not statistically different from the results obtained from stage 4 (Table S3), suggesting the extension of the pre-anoxic period did not further enhance the reactor performance in denitrification.

Collectively, we found the aeration pattern change (duration of idle period), airflow change (0.5–1 L-air/min), and pre-anoxic period duration (0−3h) change had little impact on the COD removal by SABR-J. The mean pH in the SABR-J effluent was 6.6 ± 0.4, 6.0 ± 0.2, 7.5 ± 0.1, and 6.7 ± 0.3 for stages 1 to 4 which favored nitrification (Fig. S4) [46]. The time course analysis within one cycle revealed that the pre-anoxic period, utilizing raw STE carbon, aided in denitrification, whereas the intermittent aeration contributed to efficient nitrification, thereby achieving maximum capacity N-removal overall. Furthermore, DO analysis reveals marginally elevated DO concentrations for the upper sensor in comparison to the lower sensor at all stages (Fig. S3). The observed discrepancy may be attributed to the oxygen transfer mechanism and the various factors upon which it is contingent [47].

Studies on OWTSs have presented N-removal across a broad spectrum, ranging from 19 % to 94 %. The higher removal performances are associated with either elevated C: N ratios (ranging from 3.4 to 12.3), external carbon utilization, or longer HRTs at which these technologies were operated. The SABR-J exhibited performance comparable to other aerated systems when operating with a low C: N ratio and the energy demands for aeration by SABR are notably reduced when employing sequencing aeration (20 % of time) compared to systems with extended aeration (100 % of time) (Table 2)[24].

3.2.2. *Polishing unit with low carbon amendment*

To enhance TN-removal performance, a SABR-Polish was integrated to SABR-J at stage 5 (Fig. 1 (a)). The addition of the SABR-Polish resulted in high effluent COD (average 78.8 ± 51.6 mg/L) observed during the 3-week start-up period (Fig. 2 (a)). The SABR-Polish was able to remove 65 % of NO_x^- in the main reactor effluent, therefore TN-removal performance of the integrated system was 80.6 % at stage 5 (Fig. 2 (b)), with the final effluent containing ammonia of 4.7 ± 2.4 mg-N/L and NO_x^- of 5.1 ± 4.3 mg-N/L (Fig. 2 (c)). The performance of SABR-Polish was comparable to that of sand filters integrated with woodchips, which have demonstrated a TN-removal efficiency >88 %, with effluent concentrations ranging between 5.3 and 8.3 mg-N/L [12].

3.2.3. *STE strength*

A unique characteristic of the onsite STE is that its composition fluctuates over time. At stage 6, higher strength STE was introduced to SABR-J to assess the system's capability in handling higher-strength STE. The modified STE was prepared using OECD synthetic media which had additional carbon and N added to the STE on alternate days in stages 6 and 7. Due to an increase in both COD and TN (C: N: 6.7), the system remained under anoxic condition for a longer period, and during the aeration period, the DO level remained mostly low (DO <0.5 mg/L from hour 4 to 6 of each hydraulic cycle; and DO <2 mg/L from hour 6 to 11); A decrease in DO level in SABR-J was observed leading to incomplete nitrification (49.5 %) and elevated ammonia (30.8 ± 19.4 mg-N/L) and COD (67.9 ± 69 mg/L) in the effluent (Fig. 2 (a) and (c)). The SABR-Polish successfully removed 8.4 ± 8 mg-N/L of NO_x^- in SABR-J effluent. In stage 6, the integrated SABR-J and SABR-Polish system achieved a TN-removal of 37.3 %. The primary N-species in the final effluent of the polishing unit was ammonia, which was not nitrified in the SABR-J.

To improve the nitrification performance of the reactor treating higher-strength STE without increasing aeration intensity and length, HRT was extended from 2 to 3 days at stage 7. With the increase in HRT, efficient nitrification (93.5 %) in SABR-J was achieved, and NO_x^- was the primary N-specie in the SABR-J effluent (14.7 ± 11.9 mg-N/L). SABR-Polish removed a further 13.1 % TN from the STE achieving an average of 9.9 ± 7.9 mg-N/L TN in the final effluent (Fig. 2 (b) and (c)).

Selected OWTSs show N-removal efficiency of within the range of 12.0–95 % (Table 2). Systems' performance depends on the design, HRT, aeration strategy, and C: N. OWTSs treating wastewater with C: N ratios ranging from 2.85 to 10 reported TN removals varying from 0 to 90 %, with higher C: N ratios leading to increased removal rates (Table 2). The C: N ratio acts as a factor instigating competition for growth among various microbial populations within the biofilm, consequently shaping its composition. Elevated carbon may cause undesirable nitrification inhibition in the global process since (1) heterotrophic bacteria dominate the biofilm and (2) oxygen diffusion is harder in immobilized

Table 2
N and COD removal performance in existing OWTSs.

System name	Aeration	HRT	N-removal	Organic removal	C: N	TN inf	T (°C)	Ref.
		d	%	%		mg-N/L		
SABR with 10 % woodchips polishing unit	< 4 mg/L	2	80.6	76	3.5	33.9–77.3	20–30	This study
Modified Septic Tank (MST)	> 2 mg/L	< 4.3	< 59	>95	9–10	64.7 ± 14.8 to 114.0 ± 29.6	17.6–21.4	[38]
Intermittent soil aeration	Yes	–	29.8–63.2	–	5.3–7.8 *	38.0 ± 7.0 to 47.0 ± 7.0	16.8–22.7	[49]
Onsite aerobic cyclic biological treatment unit	Yes 2.3 ± 1 mg/L	1.25	19–81	90–98	7–8.6 *	23.1 ± 4.4 & 28.1 ± 7.3	27.7 ± 1.1	[50]
SWIS	Yes	–	73.1–94.0	>95.7	2–12.3	39.5–242.4	18–29 **	(([28]; [26])
AdvanTex, Biomicrobic FAST, Peat filter, Sand filter	–	–	0–47	91–99	1.9–6.0 *	42–65	–	[51]
Advanced soil based OWTS	–	–	4.8–27	97.1–99.3	3.6 *	9	20.0 ± 0.7	[52]
MSL and SWIS	–	–	73.6	93.4	2.2–6.3	22.2–34.2	–	[53]
SWIS	–	–	69.7- 86.6	5.7–95.8	5.6–9.7	< 47.4 ± 19 (TKN)	10–32.8	([54]; [27]; [55])

* Based on BOD_5.
** Not reported in Jing Pan et al., [28,56].

biomass. Denitrification is also impacted by C: N and ratio of 7.1 ± 0.8 is needed for complete denitrification in a single reactor working with anoxic/oxic (A/O) process [48]. Studies on onsite N-removal using SWIS, MST and advanced OWTS reported an COD removal of 74–99 % (Table 2).

3.2.4. Carrier types

Alternative carriers were used to pack SABR-K at stage 5 to study the impact of carrier's type on biofilm formation and N-removal. The average COD and TN-removal were 71.5 % and 28.2 % by the SABR-K (Fig. 2 (a) and (b)). Statistical analysis (*t*-Test) showed no statistical difference of COD removal (p-value = 0.79), TN-removal (p-value = 0.46), or nitrification (p-value = 0.87) between the two SABRs (Table S3). At stage 6, when the STE strength was increased, nitrification performances of 65.9 % (SABR-K) and 46.9 % (SABR-J) were achieved (Fig. 2 (c)). There was no statistically significant difference in TN-removal (t-Test p-value 0.47), COD removal and nitrification efficiency (MW p-value 0.32 and 0.28) by SABR-J and —K during this period. In stage 7, SABR-J and —K achieved >90 % COD removal. With the HRT increase (to 3 d) nitrification of 93.5 % and 92 % and TN-removal of 74.5 % and 73.5 % were achieved in SABRs-J and —K respectively. The TN-removal efficiency for SABR-J and —K were not statistically different (t-Test p-value: 0.83). Nitrification performance treating higher strength STE was improved in both SABR-J and —K at stage 7 with extended HRT (3 days). The N and COD removal performances of SABR-J and —K were within the range of OWTSs (Table 2). Extended HRT (2–8 d) and elevated C: N (> 5.3) enhance OWTSs efficiency especially for N-removal [28,52,56].

3.3. Pilot-scale SABR

Pilot-scale SABR system consisting of a SABR-Pilot-K, and a polishing unit (Pilot-Polish), was started up and operated with optimized conditions of stage 5 (4 h pre-anoxic, 7 aeration cycles; 2d HRT). The SABR-polish started operation 30 days after SABR-Pilot-K start-up. The COD removal and nitrification were initiated after a week following start-up. The SABR-Pilot-K achieved 79.8 % COD removal from STE (Fig. 3 (a)). The Pilot-Polish initially had a high COD concentration (500 mg/L) which gradually decreased to an average of 100 mg/L which was due to carbon leaching from the fresh oak woodchips. COD removal in the SABR-Pilot-K was not statistically different from the SABR-J at stage 5 (MW p-value 0.62). SABR-Pilot-K achieved 32 % TN-removal (TN_{eff}: 16.7 ± 7.8 mg-N/L). An additional 34.3 % TN-removal from STE was achieved in the Pilot-Polish containing 10 % woodchips (TN_{eff}: 7.7 ± 5.5 mg-N/L) (Fig. 3(b)). The SABR-Pilot-K with SABR-Polish achieved <10 mg-N/L of TN in effluent. The SABR-Polish denitrified NO_x^- in SABR-Pilot-K to 0.4 ± 1 mg-N/L. TN-removal and nitrification in the pilot-scale SABR were not statistically different from the SABR-J at stage 5 (t-Test p-value: 0.48 and MW p-value: 0.51). The effluent pH of the SABR-pilot and Pilot-Polish fluctuated near neutral values (6–7) and agreed with bench-scale SABR data (Fig. S5(a)). Alkalinity in STE was sufficient to achieve nitrification and pre-anoxic denitrification

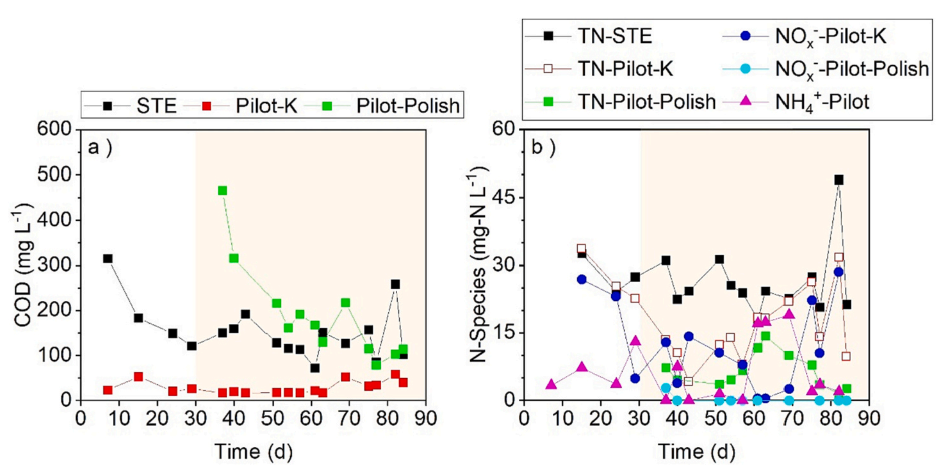

Fig. 3. (a) COD, and (b) N-species concentration in SABR-Pilot-K and the polishing unit. *Shaded area shows data after start-up of Pilot-polish.

contributed to recovering alkalinity avoiding pH drop in SABR-Pilot-K (residual alkalinity >50 mg/L as $CaCO_3$) (Fig. S5(b)) [46].

3.4. Microbial abundance and distribution change

At the end of stage 5, MLSS and biofilm samples collected from SABR-J and —K were used to calculate the biomass concentration in the SABR-J and SABR-K. The total bacterial cell number was used to calculate dry mass based on DNA concentrations measured [57,58]. Compared to MLSS concentrations of 287 mg/L and 133 mg/L, the dry solid weight of SABR-J (978 mg/L) and SABR-K (1031 mg/L) suggests the majority of biomass was accumulated in the biofilm (Fig. S6 (a) and (b)).

Functional genes involved in N-removal showed diverse distribution patterns in MLSS and biofilm in addition to woodchips samples from SABRs during stages 4 (SABR-J), 5 and 7 (SABR-J and SABR-J-Polish). The abundance of 16S rRNA remained constant during stages 4 and 5 and increased an order of magnitude in stage 7 for MLSS and remained in the range of 0.3–1.3 $\times$ 10^{11} copies/mL for biofilm. The number of total bacteria in biofilm was two orders of magnitude higher than in MLSS in stages 4 and 5, however, by stage 7 total bacteria in biofilm was comparable to MLSS (0.2 $\times$ 10^{11} vs. 1.6 $\times$ 10^{11} copies/mL) (Fig. 4). The abundance of *amoA*-AOB was stable in biofilm (stages 4, 5, and 7) and in the range of 0.3–3.2 $\times$ 10^{7} copies/mL which was in agreement with the data reported in samples collected from WWTPs [59–62] and advanced OWTSs (Table S4). While the abundance of *amoA*-AOB increased two orders of magnitude in MLSS from stage 4 (2.0 $\times$ 10^{5} copies/mL) to stage 7 (8.8 $\times$ 10^{7} copies/mL),it remained lower than in conventional activated sludge (CAS) systems (3.73 $\times$ 10^{8}–9.05 $\times$ 10^{10} copies/L of sludge). The gene *amoA*-AOA was comparable in biofilm and MLSS during stage 4 (0.3–2.7 $\times$ 10^{4} vs. 0.5 $\times$ 10^{4} copies/mL) and it decreased to levels below the detection limit (219 copies/μL) in stage 5, and therefore *amoA*-AOB (relative abundance <0.1 %) was the major nitrifier in the SABR-J. The number of *amoA*-AOA copies in CAS can vary greatly, from undetectable levels to a high of 7.4 $\times$ 10^{8} copies/mL sludge. An incongruence between influent ammonia concentration and the *amoA*-AOA gene has been observed by Limpiyakorn et al. [63], indicating higher gene copy numbers in system treating low ammonia (<10 mg-N/L) as compared to higher ammonia of 36.1–422.3 mg-N/L [63].

The nitrite reductase enzyme, encoded by either the *nirK* or *nirS*

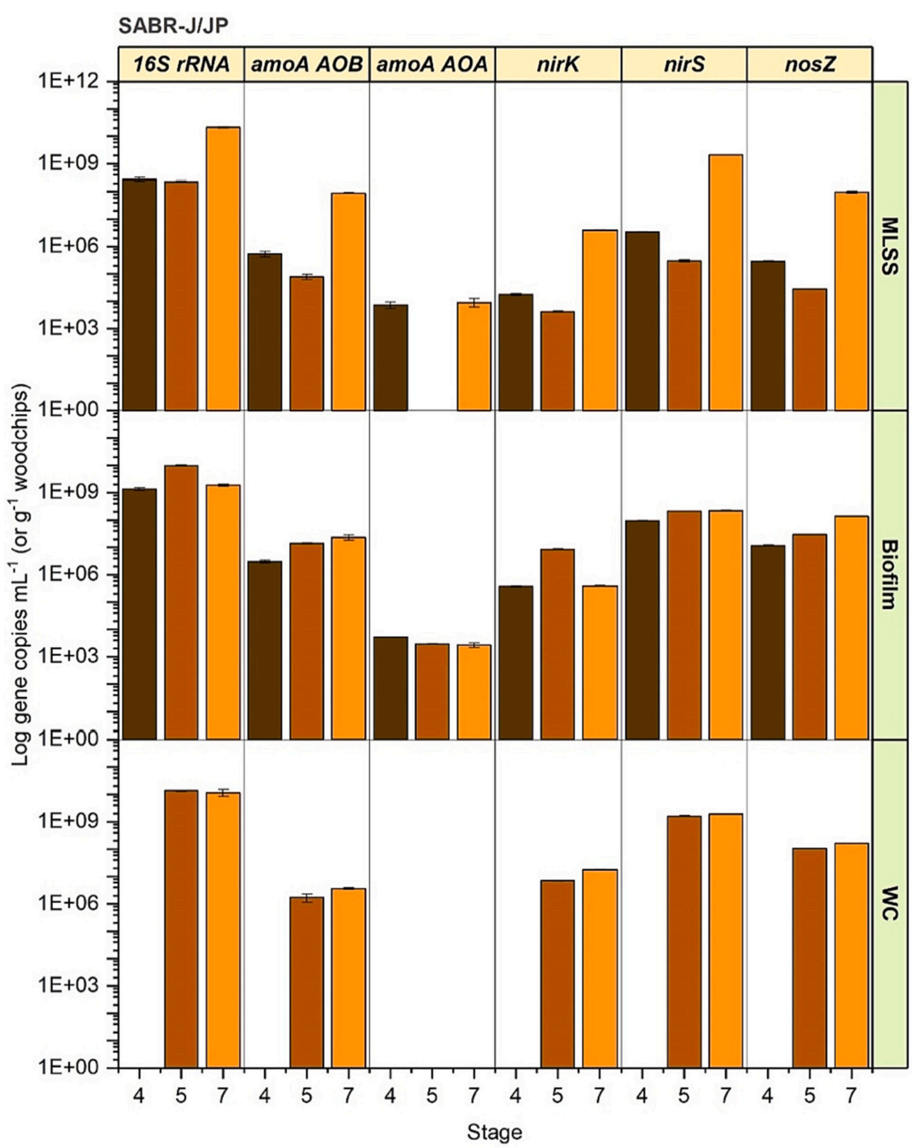

Fig. 4. Log copies numbers/mL of SABR-J and -Polish (horizontal panel represents target functional genes studied and vertical panel represents sample type. Biofilm samples were collected from SABR-J biocarriers, and numbers are normalized to the volume of reactor. Biomass from woodchips from SABR-Polish are labeled as WC and numbers are presented per gr of woodchips).

genes (containing copper or cytochrome *cd*1) catalyzes the transformation of nitrite into nitric oxide (NO). The *nosZ* gene (encoding nitrous oxide reductase) is quantified to study the process of N-removal completion to nitrogen gas [64,65]. The abundance of *nirK* gene increased in MLSS (4×10^3–4×10^6 copies/mL) and was stable in biofilm (4.0–8.6 $\times 10^6$ copies/mL) while *nirS* gene abundance was higher by two orders of magnitudes and ranged 0.9–2.2 $\times 10^8$ copies/mL in the biofilm reflecting the change in operating conditions (Fig. 4).

The ratio of nitrite reductase ($\sum$*nir*: *nirS* + *nirK*) to nitrous oxide reductase ($\sum$*nir/nosZ*) was calculated. The $\sum$*nir/nosZ* ratio served as a holistic measure of the nitrous oxide production potential [66]. To determine if SABR selected more *nirS* or *nirK*, the ratio of *nirK/nirS* was also calculated [67]. The *nirK/nirS* ratio was 0.1–1 $\times 10^{-2}$ which was within the range of biotrickling filters and continuous flow biofilter (Table S4). The relative abundance of *nirS* was the highest among all the genes, ranging from 0.2 to 1.4 % of total bacteria. Compared to *nirK* in SABR-J, the *nirS* was more abundant (2–3 orders of magnitude) which is congruent with previous studies (Table S4) [68]. The *nosZ* abundance, in the range of 0.1–1.4 $\times 10^8$ copies/mL in biofilm, was 1–3 orders of magnitude more than in MLSS. The *nosZ* abundance was within the reported range in OWTSs (9.1 to 10^6 copies/mL) and in WWTPs (6.6×10^5 to 3.5×10^8 copies/mL) (Table S4) [61,69]. In biofilm, $\sum$*nir/nosZ* was in range of 1.7 to 10, within 1–5 % of a lower range of this ratio for constructed wetlands, municipal WWTPs and in 20 % lower range of a biofilter with woodchips denitrification (Table S4) [70,71].

The abundance of 16S rRNA was stable in the range of 2–3.9 $\times 10^{11}$ copies/g of woodchips. The level of the *nirS* gene was 2–3 orders of magnitude higher than the *nirK* gene consistent with a previous study that the *nirS*-containing denitrifiers were dominant denitrifiers in woodchips denitrification column [72]. The $\sum$*nir*/16S rRNA was in the range of 5–8.3 $\times 10^{-3}$ and was in agreement with previous woodchip denitrification column studies (0.6–1.6 $\times 10^{-2}$) and was lower than in other woodchip denitrification systems treating agricultural runoff (0.1–0.8) (Table S4) [73]. The $\sum$*nir/nosZ* ratio in woodchips from SABR-Polish was in the range of 12–15.4 and higher than in the main reactors biofilm but was in the lower range of reported numbers (0.05–800) in other woodchip bioreactors, municipal WWTP, and constructed wetlands (Table S4)[70]. Lower $\sum$*nir/nosZ* potentially lead to more nitrogen gas production rather than intermediates of denitrification.

The abundance of the 16S rRNA gene in the biofilm of SABR-K was an order of magnitude higher than in MLSS (Fig. S7). The relative abundance of *amoA*-AOB, *nirK*, and *nirS* to total bacteria in SABR-K biofilm was 0.28–3.8, 0.6–3.4, and 0.3–1.6 times these genes in SABR-J biofilm (Fig. S7). The $\sum$*nir*/16S rRNA was in the range of 2.9–4.8 $\times 10^{-3}$ and $\sum$*nir/nosZ* was in the range of 2.6 to 3.8 which was lower than in SABR-J. In stage 7, the levels of *nirS*, *nirK*, and *nosZ* were comparable, whereas in stage 5, the numbers were 2 orders of magnitude higher in biofilm which is in agreement with SABR-J results.

The abundance of 16S rRNA (3.20×10^9 copies/mL) in SABR-Pilot-K was lower than the range of SABR-J and —K (0.1–1.3 $\times 10^{11}$ copies/mL). The *amoA*-AOB gene in SABR-Pilot-K was in the lower range of SABR-J and —K (5.2×10^5–3.2×10^7 copies/mL). The *nirS/nirK* was in the range of bench-scale SABRs in this study (0.1–5.7 $\times 10^2$). $\sum$*nir/nosZ* range of 4 to 8.7 for the Pilot-K and Pilot-Polish and was similar to bench-scale SABRs (1.7–10). The abundance of the studied genes in the pilot system closely matched that of SABR-J and SABR-Polish, which explains their similar removal efficiencies.

4. Conclusion

The application of SABRs has proven to be an effective method for removing nitrogen from wastewater. Not only does it improve the DO profile for nitrification, but it also creates alternating aerobic and anaerobic conditions that are ideal for denitrification. This results in the efficient removal of COD, NH_4^+-N, and TN. The success of SABRs in removing these pollutants is not dependent on the strength of the influent or the type of carrier used in the system. The implementation of SABRs can be a valuable solution, particularly in areas with limited land space or shallow groundwater tables. Two key factors that have an impact on the N-removal performance of the system are the aeration pattern (DO level and length of pre-anoxic conditions) and the composition of the wastewater (C: N ratio). In addition, the use of a polishing unit (such as 10 % woodchips) in the SABR design can improve N-removal from wastewater with dynamic compositions. The slight HRT adjustment also gives SABRs the flexibility to treat high- and low-strength domestic wastewater, making it a versatile solution for N-removal from onsite wastewater.

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CRediT authorship contribution statement

Sarah Lotfikatouli: Writing – original draft, Visualization, Validation, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Qi Pan:** Validation, Investigation. **Mian Wang:** Writing – review & editing, Investigation. **Frank M. Russo:** Supervision, Conceptualization. **Christopher J. Gobler:** Resources, Project administration. **Xinwei Mao:** Writing – review & editing, Supervision, Resources, Project administration, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jwpe.2024.105132.

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